

NHEERL Publications Available Online October 15-21, 2018

	Patel, A., J. Jagai, L. Messer, C. Gray, K. Rappazzo, S. Deflorio-Barker and D. Lobdell.	Danelle Lobdell,			
ORD-021646	Associations between environmental quality and infant mortality in the United States, 2000-2005. ARCHIVES OF PUBLIC HEALTH.	ord,	nheerl,	Peer	SHC 2.62.5;
Advance Notification	https://doi.org/10.1186/s13690-018-0306-0	ephed, eb	Reviewed		SHC 2.64.7

Cleared in STICS 6/5/2018. Now published.

Impact/Purpose Statement

This study addresses research questions under Sustainable and Healthy Communities (Project 2.64 Indicators, Indices & Report on the Environment and Project 2.62 Community Public Health & Well-Being). The National Health Environmental Effects Research Laboratory in the Environmental Public Health Division, Epidemiology Branch is currently engaged in research aimed at developing a measure that estimates overall environmental quality at the county level across the U.S. spanning the years 2000 – 2005 called the Environmental Quality Index (EQI). This work is being conducted for the purpose of learning more about how various environmental factors contribute in concert to health disparities in low-income, underrepresented minority and vulnerable populations, and to better estimate the total environmental and social context to which humans are exposed.

Product Description/Abstract

Background The United States (U.S.) suffers from one of the highest infant mortality rates among the world's developed nations and there is considerable racial/ethnic disparity in these rates. Prior studies consider associations between individual environmental exposures and infant mortality risk, but exposure is limited to singular environmental factors. We utilize the Environmental Quality Index (EQI), a measure of cumulative environmental exposure across U.S. counties from 2000-2005, to investigate associations between ambient environmental quality and infant mortality. **Methods** We linked 2000-2005 infant birth and death data from the U.S. Centers for Disease Control and Prevention to the EQI (n=22,702,529). We utilized multi-level logistic regression models to estimate associations between increasing quartiles of county-level overall and environmental domain-specific quality and individual-level infant mortality. We controlled for rural-urban status (RUCC1: urban, metropolitan; RUCC2: urban, non-metropolitan; RUCC3: less populated; RUCC4: thinly populated), maternal age, maternal education, marital status, infant sex, and stratified on maternal race/ ethnicity. Additionally, we estimated associations for linear combinations of environmental quality and rural-urban status. **Results** We found that independent and combined effects of environmental quality and rural-urban status on infant mortality varied across domain and by race/ethnicity. Increasing quartiles of EQI and sociodemographic index (poorer quality) were associated with decreased infant mortality odds among Non-Hispanic whites (OR and 95% CI: Q: 0.84[0.80,0.89]). For Non-Hispanic blacks and Hispanics, some increased odds were observed. Poorer air quality was monotonically associated with increased odds among Non-Hispanic whites (Q4: 1.05[0.99,1.11]) and blacks (Q4: 1.09 [0.9,1.31]). Rural status was strongly associated with infant mortality risk among Hispanics (RUCC4-Q4: 1.36[1.04,1.78]; RUCC1-Q4: 1.04[0.92,1.16], ref. RUCC1-Q1). **Conclusion** We observed a mix of positive, negative, and null associations between

measures of overall and domain-specific environmental quality and infant mortality. Associations varied across domain and race/ethnicity. Our study corroborates prior research on the impact of air pollution on infant mortality and demonstrates the need for further research into measures of socioeconomic deprivation across the rural-urban continuum.

	Baxter, L., K. Dionisio, P. Pradeep, K. Rappazza and L. Neas. Human exposure factors as potential determinants of the heterogeneity in city-specific associations between PM2.5 and mortality.	Lisa Baxter,	
	JOURNAL OF EXPOSURE SCIENCE and ENVIRONMENTAL	ord,	
ORD-	EPIDEMIOLOGY.	nheerl,	Peer
023330	https://doi.org/10.1038/s41370-018-0080-7	rpcs	Reviewed A-E PEP-1.4

Cleared in STICS 9/25/2017. Now available online.

Impact/Purpose Statement

This study examined the role of exposure differences in the heterogeneity of PM2.5 mortality effect estimates across the U.S., finding that factors related to infiltration and home heating fuel were significant effect modifiers to the PM2.5-mortality relationship. Areas with housing characteristics linked to increased infiltration rates or where fuel use is of a particular kind may be exposed to higher levels of outdoor pollutants and therefore be more vulnerable. National standards may not protect everyone equally, with some populations affected more than others. Determining potential factors that can cause increases in adverse PM2.5 -health effects may help identify at-risk populations and develop actions that can reduce risk.

Product Description/Abstract

Multi-city population-based epidemiological studies of short-term fine particulate matter (PM2.5) exposures and mortality have observed heterogeneity in risk estimates between cities. Factors affecting exposures, such as pollutant infiltration, which are not captured by central-site monitoring data, can differ between communities potentially explaining some of this heterogeneity. This analysis evaluates exposure factors as potential determinants of the heterogeneity in 312 core-based statistical areas (CBSA)-specific associations between PM2.5 and mortality using inverse variance weighted linear regression. Exposure factor variables were created based on data on housing characteristics, commuting patterns, heating fuel usage, and climatic factors from national surveys. When survey data were not available, air conditioning (AC) prevalence was predicted utilizing machine learning techniques. Across all CBSAs, there was a 0.95% (Interquartile range (IQR) of 2.25) increase in non-accidental mortality per 10 µg/m3 increase in PM2.5 and significant heterogeneity between CBSAs. CBSAs with larger homes, more heating degree days, a higher percentage of home heating with oil had significantly ($p < 0.05$) higher health effect estimates, while cities with more gas heating had significantly lower health effect estimates. While univariate models did not explain much of heterogeneity in health effect estimates ($R^2 < 1\%$), multivariate models began to explain some of the observed heterogeneity ($R^2 = 13\%$).

	Corton, C., A. Williams, and C. Yauk. Using a Gene Expression Biomarker to Identify DNA Damage-Inducing Agents in Microarray Profiles.	Chris Corton,		
	ENVIRONMENTAL AND MOLECULAR	ord,		
ORD-	MUTAGENESIS	nheerl,	Peer	
025118	https://doi.org/10.1002/em.22243	istd,sbb	Reviewed	CSS16.01.01

Cleared in STICS 4/14/2018. Now available online

Impact/Purpose Statement

High-throughput transcriptomic technologies are increasingly being used to screen environmental chemicals in vitro to provide mechanistic context for regulatory testing. The TGx-DDI biomarker is a 64-gene expression profile generated from testing 28 model chemicals or treatments (13 that cause DNA damage and 15 that do not) in human TK6 cells. While the biomarker is very accurate at predicting DNA damage inducing (DDI) potential using the nearest shrunken centroid method, the broad utility of the biomarker using other computational methods is not known. Here, we determined whether the biomarker used with the Running Fisher test, a nonparametric correlation test, could accurately identify DDI agents in microarray comparisons derived from human cell lines treated with chemicals vs. matched controls.

Product Description/Abstract

High-throughput transcriptomic technologies are increasingly being used to screen environmental chemicals in vitro to provide mechanistic context for regulatory testing. The TGx-DDI biomarker is a 64-gene expression profile generated from testing 28 model chemicals or treatments (13 that cause DNA damage and 15 that do not) in human TK6 cells. While the biomarker is very accurate at predicting DNA damage inducing (DDI) potential using the nearest shrunken centroid method, the broad utility of the biomarker using other computational methods is not known. Here, we determined whether the biomarker used with the Running Fisher test, a nonparametric correlation test, could accurately identify DDI agents in microarray comparisons derived from human cell lines treated with chemicals vs. matched controls. In TK6 cells the methods could readily differentiate DDI and non-DDI compounds with balanced accuracies of 87% - 97%, depending on the threshold for determining DDI positives. The methods were also useful for identifying DDI agents in the metabolically competent hepatocyte cell line HepaRG (accuracy = 90%) but not in HepG2 or hepatocytes derived from embryonic stem cells (60% and 80%, respectively). In addition, the methods accurately predicted DDI when the gene expression changes were derived using the nCounter technology (accuracy = 89%). The contribution of individual genes to the significance of the correlations was determined. We found: 1) not all genes contributed equally to the correlations, with three genes in particular (PCDH8, SEMG2, TNFRSF17) rarely altered across the comparisons, 2) the minimal overlap in genes between the biomarker and the individual comparisons required for significant positive correlation was 5 genes, but usually was much higher, and 3) different sets of genes in the biomarker can by themselves contribute to the significant correlations. Overall, these results demonstrate the utility of the biomarker used with different computational methods to accurately predict DDI agents.

Catron, T., S. Keely, N. Brinkman, T. Zurlinden, C. Wood, J. Wright, D. Phelps, E. Wheaton, A. Kvasnicka, S. Gaballah, R. Lamendella and T. Tal. Tamara
 Host development toxicity of BPA and BPA alternatives is inversely related to microbiota disruption in zebrafish. TOXICOLOGICAL SCIENCES <https://doi.org/10.1093/toxsci/kfy261> nheerl, Peer
 ORD- 025268 istd Reviewed CSS17.01.01

Cleared in STICS 2/21/2018. Now available online.

Impact/Purpose Statement.

Intestinal microbes are thought to play important roles in early development. It has been proposed that microbiota can bioactivate or detoxify environmental chemicals and mediate health effects of chemical exposure. Bisphenol A is a widespread environmental contaminant found in a variety of consumer products. Due to concerns over toxicity, bisphenol A has been replaced with structurally similar alternative chemicals. Using zebrafish as a model, this work shows that exposure to bisphenol A, bisphenol F, and bisphenol S, the three chemicals identified to be the least toxic to the developing zebrafish, results in significant disruption of microbial community structure and function. Exposure to bisphenol B and bisphenol AF, the two most developmentally toxic chemicals, does not alter microbiota structure. Exposure to bisphenol A and alternatives does not alter locomotor activity, an endpoint used to assess neurotoxicity. To our knowledge, this is the first study to show that microbiota disruption is, in general, inversely related to host developmental toxicity and estrogenic activity of bisphenol A and alternatives. These findings indicate that exposure to bisphenol A and certain structurally similar alternatives alter microbial communities during early life and demonstrate novel chemical-microbe interactions that may add context to current hazard identification strategies.

Product Description/Abstract

Background: Host-associated microbiota can biotransform xenobiotics, mediate health effects of chemical exposure, and play important roles in early development. Bisphenol A (BPA) is a widespread environmental chemical that has been associated with adverse endocrine and neurodevelopmental effects, some of which may be mediated by microbiota. Growing public concern over the safety of BPA has resulted in its replacement with structurally similar alternatives. Objectives: We evaluated whether BPA and BPA alternatives alter microbiota and lead to secondary adverse effects in zebrafish. Methods: Zebrafish were developmentally exposed to BPA, Bisphenol AF (BPAF), Bisphenol B (BPB), Bisphenol F (BPF), or Bisphenol S (BPS). At 10 days post fertilization (dpf), toxicity assessments were completed and 16S rRNA gene sequencing was performed to evaluate potential chemical-dependent shifts in microbial community structure and function. A standard light/dark behavioral assay was used to assess locomotor activity. Results: Based on developmental toxicity at 10 dpf, a range of potencies was observed: BPAF > BPB > BPA > BPF > BPS. Analysis of 16S rRNA gene sequencing data showed significant concentration-dependent disruption of microbial community structure and enrichment of many microbial functions with exposure to BPS, BPF, and BPA, but not BPB and BPAF. Interestingly, microbial disruption was largely inversely related to host developmental toxicity and estrogenicity. Exposure to BP analogues did not cause neurobehavioral toxicity at 10 dpf. Conclusions: Our findings indicate that some BP analogues disrupt host microbiota early in life and demonstrate novel chemical-microbiota interactions that may add context to current hazard identification strategies.

	Barnhart, B., H. Golden, J. Kasprzyk, J. Pauer, C. Jones, K. Sawicz, N. Hoghooghi, M. Simon, B. Mckane, P. Mayer, A. Piscopo, D. Ficklin, J. Halama, P. Pettus, and B. Rashleigh. Embedding co-production and addressing uncertainty in watershed modeling decision-support tools: Successes and challenges. ENVIRONMENTAL MODELLING & SOFTWARE. Elsevier Science, New York, NY,	Brad Barnhart, ord, nheerl, wed, eeb	Peer Reviewed	SSWR5.01A
ORD-024960	https://www.sciencedirect.com/science/article/pii/S1364815218300781			

Cleared in STICS 1/22/2018. Now published.

Impact/Purpose Statement

This journal article provides a roadmap for producing effective decision-support tools that utilize mechanistic watershed models. We emphasize the importance of co-production—that is, the co-development of methods, tools, and results through close interactions between technical experts and stakeholders—as well as inclusion of uncertainty. The review uses literature-based case study examples to demonstrate each step of the roadmap and highlight successes and challenges associated with current and past decision-support tools. This work will be submitted to Environmental Modelling & Software and will be generally applicable to technical experts and stakeholders that intend to produce or utilize watershed model decision-support tools.

Product Description/Abstract

Decision-support tools (DSTs) are often produced from collaborations between technical experts and stakeholders to address environmental problems and inform decision making. Studies in the past two decades have provided key insights on the use of DSTs and the importance of bidirectional information flows among technical experts and stakeholders – a process that is variously referred to as co-production, participatory modeling, structured decision making, or simply stakeholder participation. Many of these studies have elicited foundational insights for the broad field of water resources management; however, questions remain on approaches for balancing co-production with uncertainty specifically for watershed modeling decision support tools. In this paper, we outline a simple conceptual model that focuses on the DST development process. Then, using watershed modeling case studies found in the literature, we discuss successful outcomes and challenges associated with embedding various forms of co-production into each stage of the conceptual model. We also emphasize the “3 Cs” (i.e., characterization, calculation, communication) of uncertainty and provide evidence-based suggestions for their incorporation in the watershed modeling DST development process. We conclude by presenting a list of best practices derived from current literature for achieving effective and robust watershed modeling decision-support tools.

	Mustafa, M., B. Barnhart, M. Babbar-Sebens and D. Ficklin. Modeling landscape change effects on stream temperature using the Soil and Water Assessment Tool. WATER. MDPI AG, Basel, SWITZERLAND, https://www.mdpi.com/2073-4441/10/9/1143/html	Brad Barnhart, ord, nheerl, wed, eeb	Peer Reviewed	SSWR 5.01A
ORD-027239				

Cleared in STICS 7/17/2018. Now published

Impact/Purpose Statement

This publication will be submitted to the journal "Water" in the special issue "Impacts of Landscape Change on Water Resources." The manuscript focuses on an improvement to a stream temperature model in the widely used watershed model SWAT (Soil and Water Assessment Tool). Specifically, the study investigates how including radiative components in addition to air temperature and hydrologic effects can improve stream temperature simulations. It also allows SWAT to simulate the changes in stream temperature due to differences in landscape cover (e.g., forested vs. agricultural subbasins). This work directly impacts model developers and SWAT users including watershed managers and the general public interested in stream temperature modeling as well as stream temperature estimates produced by watershed models.

Product Description/Abstract

Stream temperature is one of the most important factors regulating fish behavior and habitat; therefore, models that seek to characterize stream temperatures and predict their changes due to landscape and climatic changes are extremely important. In this study, we extend a mechanistic stream temperature model within the Soil and Water Assessment Tool (SWAT) by explicitly incorporating radiative flux components to more realistically account for radiative heat exchange. The extended stream temperature model is particularly useful for simulating the impacts of landscape and land use change on stream temperatures using SWAT. The extended model is tested for the Marys River, a western tributary of the Willamette River in Oregon, and the results are compared with observed stream temperatures as well as previous model estimates (without radiative components) for different spatial locations within the Marys River watershed. Using the radiative stream temperature model, stream temperature simulations in forested subbasins are approximately 4 °C lower than agricultural subbasins in the Marys River watershed. However, more noise was generated in the radiative model due to the inclusion of highly variable radiative forcing components. The model works at a daily time step, and further research should investigate modeling at hourly timesteps to further improve the temporal resolution of the model. In addition, other watersheds should be tested using this model to determine its applicability in different climates, landscapes, and land use regimes.

	Henkel, S., and W. Nelson. Assessment of spatial patterns in benthic macrofauna of the U.S. west coast continental shelf. JOURNAL OF BIOGEOGRAPHY.	Walt Nelson, ord, nheerl, wed, pceb	Peer Reviewed	N/A
ORD-024456	Blackwell Publishing, Malden, MA, USA, https://doi.org/10.1111/jbi.13451			

Cleared in STICS 12/15/2017. Now available online.

Impact/Purpose Statement

In order to provide optimal approaches to interpretation of regional scale monitoring data such as the National Aquatic Resource Surveys, research assessed whether currently described marine biogeographic boundaries for the U.S. West coast apply to shelf macrofauna. The environmental drivers that were most associated with species differences among biogeographic regions was also assessed. Benthic infauna on the continental shelf from the Strait of Juan de Fuca in Washington to the California-Mexico border were sampled together with a range of sediment and water column metrics. The biographic boundaries proposed under the Marine Ecoregions of the World schema are more closely aligned with shelf fauna distributions than those developed based on west coast rocky intertidal communities. However, the proposed province boundary at Cape Mendocino is not apparent in the shelf macrofauna, and a transition appears to occur closer to the Oregon-California border. Further, the shelf fauna indicate the Channel Islands should be a separate subregion from the mainland southern California Bight.

Product Description/Abstract

Aim. We assessed whether currently described marine biogeographic boundaries apply to shelf macrofauna and which environmental drivers were most associated with species differences among regions. **Location.** Pacific coast of North America from the Strait of Juan de Fuca in Washington to the California-Mexico border. **Methods.** Grab samples were collected from soft sediment 28-138 m deep. Spatial patterns of species richness, diversity, and abundance were examined in relation to latitude and environmental parameters. Analyses of latitudinal distribution patterns of individual species were combined with multivariate analyses of community composition to determine biogeographic and habitat boundaries for mid-depth continental shelf macrofauna. **Results.** Community parameters significantly decreased with increasing latitude, primarily between 32 and 40° N. There were positive associations of species richness and diversity with bottom dissolved oxygen and temperature, but not salinity or depth, and of richness, diversity, and abundance with upwelling index, sediment percent fines, and TOC (<2%). Assessment of individual species ranges found major faunal transitions at 33-34°, 37°, 44°, and 46-47°. Major assemblage differences were found at 34.5°, and 42°. Within each latitudinal region, significantly different macrofauna communities were found in sediment with very low percent fines. **Main Conclusions** The biographic boundaries proposed under the Marine Ecoregions of the World schema are more closely aligned with shelf fauna distributions than those developed based on west coast rocky intertidal communities. However, the proposed province boundary at Cape Mendocino is not apparent in the shelf macrofauna, and a transition appears to occur closer to the Oregon-California border. Further, the shelf fauna indicate the Channel Islands should be a separate subregion from the mainland southern California Bight. Multivariate community analyses minimizing the impact of rare species appeared more useful in determining macrofaunal community biogeographic boundaries than

methods involving analysis of individual species range endpoints, which are strongly influenced by uncommon species.

**NHEERL Publications Accepted by Journal
October 15-21, 2018**

No items to report.

NHEERL Publications Cleared in STICS

October 15-21, 2018

	Larsen, A., S. Yang, B. Reich, and A. Rappold. A spatial causal analysis of wildland fire- contributed PM2.5 using numerical model output. JOURNAL OF THE AMERICAN STATISTICAL ASSOCIATION. American Statistical Association, Alexandria, VA, USA,	Ana Rappold, ord, nheerl, ephd, crb	Peer Reviewed	A-E PEP-4.3
ORD- 028408				

Impact/Purpose Statement

We propose a method for separating estimate of fire-contributed PM2.5 from non-fire PM2.5 concentrations using a novel causal inference framework and bias-adjusted computer simulations of PM2.5 under counterfactual scenarios.

Product Description/Abstract

Wildland fire smoke contains hazardous levels of fine particulate matter (PM2.5), a pollutant shown to adversely effect health. Estimating fire attributable PM2.5 concentrations is key to quantifying the impact on air quality and subsequent health burden. This is a challenging problem since only total PM2.5 is measured at monitoring stations and both fire-attributable PM2.5 and PM2.5 from all other sources are correlated in space and time. We propose a framework for estimating fire-contributed PM2.5 and PM2.5 from all other sources using a novel causal inference framework and bias-adjusted chemical model representations of PM2.5 under counterfactual scenarios. The chemical model representation of PM2.5 for this analysis is simulated using Community Multi-Scale Air Quality Modeling System (CMAQ), run with and without fire emissions across the contiguous U.S. for the 2008-2012 wildfire seasons. The CMAQ output is calibrated with observations from monitoring sites for the same spatial domain and time period. We use a Bayesian model that accounts for spatial variation to estimate the effect of wildland fires on PM2.5 and state assumptions under which the estimate has a valid causal interpretation. Our results include estimates of absolute, relative and cumulative contributions of wildfires smoke to PM2.5 for the contiguous U.S. Additionally, we compute the health burden associated with the PM2.5 attributable to wildfire smoke.

	Lehmann , D., and W. Williams. Development and Utilization of a Unique In Vitro Antigen Presentation Co-culture Model for Detection of Immunomodulating Substances.. TOXICOLOGY IN VITRO. Elsevier Science Ltd, New York, NY, USA,	David Lehmann, ord, nheerl, ephd, cib	Peer Reviewed	CSS 2.5.1
ORD- 014945				

Impact/Purpose Statement

Pressure is mounting for the development and utilization of alternative in vitro methods for identifying potential immunotoxicants. In line with these efforts, we developed a novel in vitro cell-based assay to evaluate effects on antigen presentation - a key step in successful immunization. This novel in vitro assay of immune function may be suitable for integration into a tiered testing battery for screening and prioritization of potential immunosuppressants.

Product Description/Abstract

Current regulatory immunotoxicity studies require the use of animal models. However, evolving regulatory requirements, the need to evaluate large numbers of chemicals efficiently and societal pressures are driving the development and utilization of alternative in vitro methods for identifying potential immunotoxicants. In line with these efforts, we developed a novel in vitro cell-based assay to evaluate effects on antigen presentation - a key step in successful immunization. In this assay, Ch27 B cells acquire and present hen egg lysozyme peptides to antigen-restricted 3A9 T cells, causing them to produce and secrete IL-2. IL-2 levels in the culture medium may be monitored to identify effects of immunotoxicant exposure on antigen uptake, processing or presentation by the Ch27 cells and on antigen recognition and IL-2 production and secretion by the 3A9 cells. IL-2 production was reduced in response to treatment with well-known immunotoxicants cyclosporin A (CYA), dexamethasone (DEX), azathioprine (AZPR), methotrexate (MOT) and benzo(a)pyrene (BAP) but was not affected by treatment with CYPH. A negative control compound mannitol (MANN) altered neither cell viability nor IL-2 levels whereas the positive control compound ammonium chloride (AMCL) reduced IL-2 production. This novel in vitro assay of immune function may be suitable for integration into a tiered testing battery for screening and prioritization of potential immunosuppressants.

	Lehmann, D., M. Batres, and W. Williams.	
ORD-	Impact of Diflubenzuron on <i>Bombus</i>	David
027438	<i>impatiens</i> Microcolony Progression.	Lehmann,
Adv.	PLoS ONE. Public Library of Science, San	ord, nheerl, Peer
Notification	Francisco, CA, USA,	ephed, cib Reviewed CSS18.04.01

Impact/Purpose Statement

Reliance on the honey bee as a surrogate organism for risk assessment performed on other bees is being challenged. Consequently, there is a need to develop validated methods for assessing toxicity in non-Apis bees including bumble bees. Using diflubenzuron as a model toxicant, we developed a protocol for initiating and monitoring bumble bee microcolonies. While our effort strengthens the foundation for use of this methodology in risk assessment, more work is required to better understand the utility of this model for risk assessment.

Product Description/Abstract

Reflecting differences in phenology and life history as well as differences in sensitivity to pesticides, reliance on the honey bee as a surrogate organism for risk assessment performed on other bees is being challenged. Consequently, there is a need to develop validated methods for assessing toxicity in non-Apis bees including bumble bees. The usefulness of microcolonies for risk assessment has not been fully investigated. Using diflubenzuron as a model test article, we monitored microcolony development from nest initiation to drone emergence using *B. impatiens*, a species relevant to North America. Microcolony progression from egg-laying to drone production was carefully monitored and photographically documented. Using this model, diflubenzuron dose-dependently inhibited drone production with an IC50 of 36.7 g/L. Microcolony food utilization mirrored brood development, peaking during the third or fourth week of this study. Compared to vehicle control-treated microcolonies, microcolonies dosed with 1 mg/mL diflubenzuron blunted food consumption and had no effect on the time to first oviposition, but the time to egg hatching and emergence of the first drone were delayed. No concentration of diflubenzuron tested altered worker survival. While our data strengthen the foundation for use of this methodology, more work is required to better understand the utility of this model for risk assessment.

	Wang, J., D. Hallinger, A. Murr, A. Buckalew, R. Lougee, A. Richard, S. Laws, and T. Stoker. High-Throughput Screening and Chemotype- Enrichment Analysis of ToxCast Phase II	Tammy		
ORD-	Chemicals Evaluated for Human Sodium-	Stoker,		
027928	Iodide Symporter (NIS) Inhibition.	ord,		
Adv.	ENVIRONMENT INTERNATIONAL. Elsevier	nheerl,	Peer	
Notification	B.V., Amsterdam, NETHERLANDS,	tad, etb	Reviewed	CSS16.01.01

Impact/Purpose Statement

The use of hNIS-HEK293T-EPA cells in the NIS-mediated iodide uptake and cell viability assays provide the U.S.EPA with a rapid approach for identifying chemicals that have the potential to disrupt a key molecular step essential for the synthesis of thyroid hormones. This manuscript demonstrates that the NIS HTS approach can identify chemicals that have the potential to inhibit the NIS and the newly developed ranking system can be used to prioritize the chemicals for further evaluation in both orthogonal assays and short-term in vivo studies. Here, we report the NIS inhibitor screening for 768 ToxCast Phase II (ph2) chemicals. The 172 chemicals that were active in the RAIU assay were ranked based on potency, effects on cell viability, and normalized to perchlorate. Some of the highest ranked chemicals included PFOS, tributyltin chloride, and triclocarban, which have been shown to disrupt the thyroid axis. The present results were then combined with the previous ph1v2 library screening results to produce two sets of binary hit-calls for 1028 unique chemicals, consisting of 273 positives exhibiting significant RAIU inhibition, and 63 positives following application of a cell viability filter. A ToxPrint chemotype-enrichment analysis identified more than 20 distinct chemical substructure features associated with each version of the NIS inhibition hit-call space. A shared set of 9 unique ToxPrint chemotypes enriched in both hit-call sets indicates strong chemotype signals that are insensitive to cell viability filters, that can help to guide SAR investigations and inform future experiments. This chemotype information and SAR for both active and inactive chemicals will be used to test predictions in the NIS screening of the E1K ToxCast library of over 800 chemicals and guide follow-up studies.

Product Description/Abstract

In support of the Endocrine Disruptor Screening Program (EDSP), the U.S. EPA's Office of Research and Development (ORD) is developing high-throughput screening (HTS) approaches to identify chemicals that may alter target sites in the thyroid hormone (TH) pathway. The sodium iodide symporter (NIS) is a transmembrane glycoprotein that mediates iodide uptake into the thyroid gland as the initial step of TH biosynthesis. Previously, we screened 293 ToxCast Phase I chemicals using a HEK293T cell line expressing human NIS in parallel radioactive iodide uptake (RAIU) and cell viability assays to identify potential environmental NIS inhibitors. Here, we expanded NIS inhibitor screening for 768 ToxCast Phase II chemicals. Following single-concentration screening (at 1×10^{-4} M with a 20% inhibition cutoff), 235 samples were further tested in multiple-concentration (1×10^{-9} - 1×10^{-4} M) format in both RAIU and cell viability assays. The 172 samples that exhibited significant RAIU inhibition were then prioritized using combined RAIU and cell viability responses, and normalized relative to the known NIS inhibitor sodium perchlorate. Some of the highest ranked chemicals, such as PFOS, tributyltin chloride, and triclocarban, have been previously reported to be thyroid disruptors. In addition, several novel chemicals were identified as potent NIS inhibitors. The present results were combined with the previous Phase I screening results to produce two sets of binary hit-calls for 1028 unique chemicals, consisting of 273 positives exhibiting significant RAIU inhibition, and 63 positives following application of a cell viability

filter. A ToxPrint chemotype-enrichment analysis identified more than 20 distinct chemical substructural features, represented in more than 60% of the active chemicals, as significantly enriched in each NIS inhibition hit-call space. A shared set of 10 chemotypes enriched in both hit-call sets indicates highly stable chemotype signals that can help to guide structure-activity relationship (SAR) investigations and inform future research.

	Kolanczyk, R., J. Denny, B. Sheedy, P. Schmieder, and M. Tapper. Estrogenic activity of multicyclic aromatic hydrocarbons in rainbow trout (<i>Oncorhynchus mykiss</i>) in vitro assays. AQUATIC TOXICOLOGY. Elsevier Science Ltd, New York, NY, USA,	Rick Kolanczyk, ord, nheerl, med, stb	Peer Reviewed	CSS18.04.01
ORD-027943 Adv. Notification				

Impact/Purpose Statement

This first report of bridging multicyclic aromatic hydrocarbons in fish demonstrates binding for a series of chemicals normally not thought of as having an affinity for the ER and further illustrates the variety of chemical structures capable of binding to the ER. In fact, 3PE shows a high binding affinity for rtER (RBA=3.0%) and high potency in the Vtg mRNA expression liver slice assay, comparable to higher affinity steroids like E1, E3, and α -E2. We were able to expand the ERES model decision tree for chemical prioritization while incorporating multicyclic aromatic hydrocarbons.

Product Description/Abstract

A representative group of multicyclic aromatic hydrocarbons (MAHC) which can be further classified as bridged-ring (bridged-MAHC) or fused-ring (fused-MAHC) were examined for their ability to interact with the estrogen receptor of rainbow trout (rtER) in a hepatic cytosolic estrogen receptor competitive binding assay (cyto rtER) and the vitellogenin (Vtg) mRNA gene activation liver slice assay. All five fused-MAHCs; naphthalene (NAFT), fluorene (FE), Fluoranthene (FAT), pyrene (PY), and 9,10-dihydroanthracene (DAC) had no estrogenic activity in the in vitro assays used. Five of the eight bridged-MAHCs; triphenylethylene (3PE), o-terphenyl (OTP), triphenylmethane (TPM), 1,1-diphenylethylene (DPE), and cis-stilbene (CSB) were estrogenic in these assays. The additional three bridged-MAHC's; trans-stilbene (TSB), tetraphenylethylene (4PE), and 4,4-di-tertbutylphenyl (DtBB) were determined to be non-binders due to isomeric configuration, solubility limitation, and possible steric hinderance. It is possible that the bridged-MAHCs bind to the rtER through a proposed aromatic-aromatic stacking (δ - δ interaction) facilitated by perpendicular ring orientation achieved through free rotation of the bridged rings. The fused-ring structures are locked in a planar configuration which doesn't allow for rotation of rings perpendicular to one another. This first report of the rtER-binding of bridged-MAHCs in fish demonstrates binding for a class of chemicals normally not thought of as having an affinity for the estrogen receptor and further supports the versatility or promiscuity of ER ligand selectivity.

	Gray, E., J. Furr, J. Conley, C. Lambright, N. Evans, M. Cardon, V. Wilson, P. Foster, and P. Hartig. A Conflicted Tale of Two Novel AR Antagonists In vitro and In vivo: Pyrifluquinazon versus Bisphenol C. TOXICOLOGICAL SCIENCES. Society of Toxicology, RESTON, VA,	Earl Gray, ord, nheerl, tad, rtb	Peer Reviewed	CSS17.01.01
ORD-027963				

Impact/Purpose Statement

Predicting the effects from in vitro to in vivo adverse effects for endocrine disrupting chemical is a major goal of the EDSP, EPA and part of the effort to reduce, refine and replace animal use with new approach methodology. Herein, we examined some of the limitations of extrapolating from in vitro androgen receptor binding and gene expression assays to adverse effects in utero on the male rat reproductive tract. These results indicate that ADME analyses must be included in attempts to extrapolate from in vitro AR assays to in vivo adverse effects.

Product Description/Abstract

Chemicals that disrupt androgen receptor function during sexual differentiation in utero induce a cascade of adverse effects on F1 male reproductive development including reduced AGD, retained nipples, agenesis of androgen-dependent organs and reproductive tract malformations. Among the chemicals that act as androgen receptor (AR) antagonists are flutamide, vinclozolin, and procymidone. Efforts are underway to use in vitro AR antagonism data to predict the adverse effects of in vivo exposure; however, the value of this approach has only been examined with a limited number of carefully selected reference chemicals. The objective of the current study is to compare the in vitro and in vivo activity of two novel AR antagonists, bisphenol C (BPC) and pyrifluquinazon (PFQ). Our results, like those in the literature, demonstrate that BPC is as potent as hydroxyflutamide in vitro, both being orders of magnitude more potent than PFQ. However, the ability of BPC to disrupt androgen-signaling in vivo has not been described. PFQ has been reported to interact with the rat AR, but not human AR, at high concentrations and to reduce androgen dependent tissue weights in the rat Hershberger assay. The objective of this study was to compare the potency of BPC to PFQ in vitro and their effects in vivo on reproductive development in male rat offspring. BPC was about 380-fold more potent than PFQ in vitro, but it was only about 2.5% as potent as PFQ in reducing AGD at birth and in inducing male reproductive tract abnormalities. In the absence of ADME models, extrapolating the effects of AR antagonists from in vitro to in vivo is an uncertain process, at best.

	Ortenzio, J., L. Degn, A. Goldstein-Plessner, J.	William	
	Mcgee, J. Navratilova, K. Rogers, R. Zucker, and	Boyes,ord,	
ORD-	W. Boyes. Determination of Silver Nanoparticle	nheerl,	Peer
028279	Dose in vitro.	tad, nb	Reviewed CSS18.02.01

Impact/Purpose Statement

This manuscript demonstrates the importance of expressing the dose for in vitro nanotoxicology studies as the amount of nanomaterials reaching the cells rather than as the concentration added to the system, because of differential dispersion and settling of different sized particles. It also demonstrates that flow cytometry can be an efficient approach to measuring the number or mass of nanoparticles absorbed by cells.

Product Description/Abstract

An important issue for interpreting in vitro nanomaterial testing is quantifying the dose delivered to target cells. Considerations include the concentration added to the culture, the proportion of the applied dose that interacts with the target cells, and the amount that is eventually absorbed by the target cells. Rapid and efficient techniques are needed to determine delivered doses. Previously, we demonstrated that TiO₂ and silver nanoparticles (AgNP) were absorbed by cells in a dose dependent manner between 1 g/ml and 30 g/ml, and were detected by light scatter using a flow cytometer. Here, we compare four potential indices of the dose of AgNP to cells, including: inductively coupled plasma -

mass spectrometry (ICP-MS); flow cytometry side scatter (SSC); and amount of silver deposited to the cell layer as estimated with both an in vitro sedimentation, diffusion and dosimetry model (ISDD) and a distorted grid (DG) model. A retinal pigment epithelial cell line was exposed to 20 nm or 75 nm citrate-coated AgNP for 24 hr. The relationships between particle sizes and internalized doses varied according to the dose metric. Twenty-four hours after exposure, the cell layer contained a greater mass of silver when treated with 75 nm AgNP than with 20 nm AgNP. When the dose was expressed as the number of particles or as the total surface area of absorbed particles, however, the reverse was true; the dose to the cells was higher after exposure to 20 than 75 nm AgNP. Flow cytometry SSC increased with dose for both sizes of AgNP, and was correlated with Ag in cells measured by ICP-MS. The rate of SSC increase was greater for 75 than for 20 nm AgNP, suggesting it could be used as an indicator of cellular dose after accounting for particle size and composition. Silver was detected by ICP-MS in re-suspended supernates of the isolated cell layer suggested that not all the silver deposited to the cell layer was absorbed by the cells. Both the ISDD and DG models overestimated the proportion of Ag absorbed into the cells, but provided more reasonable estimates when compared against the total Ag deposition, i.e. mass of silver in the cells plus the resuspended, unabsorbed Ag from the cell layer. ICP-MS indicated the mass of silver in cells from AgNP treatment, but not whether the Ag was in the form of particles or dissolved ions. Deposition models predicted deposition of AgNP to the cell layer, but not cellular uptake. Flow cytometry SSC was correlated to cellular uptake of particle-form AgNP and could be calibrated against ICP-MS to indicate mass of cellular uptake. Therefore, a combination of approaches may be required to accurately understand cellular dosimetry of in vitro nanotoxicology experiments. In summary, cellular dosimetry is an important consideration for nanotoxicology experiments, and not necessarily related to the applied dose.

	Adetona, A., W.K. Martin, S. Warren, N. Hanley, O. Adetona, J.(. Zhang, C. Simpson, M. Paulsen, S. Rathbun, J. Wang, D. DeMarini, and L. Naeher.		
	Urinary Mutagenicity as a Biomarker of Occupational Smoke Exposures of Wildland Firefighters Performing Prescribed Burns.	David DeMarini , ord,	
ORD- 028485	INHALATION TOXICOLOGY. Taylor & Francis, Inc., Philadelphia, PA, USA,	nheerl, istd, gctb	Peer Reviewed N/A

Impact/Purpose Statement

Results from this study suggest that healthy, non-smoking wildland firefighters are exposed to genotoxic compounds during prescribed burning. Urinary mutagenicity may serve as a suitable measure of occupational smoke exposures among this worker population when exposure levels are likely higher than what was observed in this study. No statistically significant cross-work shift increases were observed in creatinine-adjusted urinary mutagenicity between burn and non-burn days. However, our results suggest that firefighters using drip-torches to light fires have potentially higher urinary mutagenicity during prescribed burns than during other work tasks. Findings from this study suggest that occupational smoke exposure, especially related to tasks involving lighting fires on prescribed burns, may contribute to systemic exposure to mutagens.

Product Description/Abstract

Background: Wildland firefighters conducting prescribed burns are exposed to a complex mixture of pollutants, requiring an integrated measure of exposure.

Objective: We used urinary mutagenicity to assess if systemic exposure to mutagens are higher in firefighters after working at prescribed burns versus after non-burn work days.

Methods: Using a repeated measures study design, we collected urine before, immediately after, and the morning after a work shift on prescribed burn and non-burn work days from 12 healthy subjects. Urines were analyzed for malondialdehyde (MDA), 8-isoprostane, 1-hydroxypyrene (OH-Pyrene), and mutagenicity in Salmonella YG1041 +S9. Particulate matter (PM2.5) and carbon monoxide (CO) measurements were collected by personal monitoring. Light-absorbing carbon of PM2.5 was measured as a surrogate for black carbon exposure. Linear mixed-effect models were used to assess cross-work shift (pre- to post-work shift) changes in urinary biomarkers.

Results: No significant differences occurred in creatinine-adjusted urinary mutagenicity across the work shift between burn days (48 samples) and non-burn day (21 samples). Firefighters lighting fires had a non-significant, 1.6-fold increase in urinary mutagenicity for burn- versus non-burn day exposures. Positive associations were found between cross-work shift (pre- to post-) changes in creatinine-adjusted urinary mutagenicity and MDA ($p = 0.0010$), OH-Pyrene ($p = 0.0001$), and mass absorption efficiency ($p = 0.2245$), respectively. No significant effect of day type or work task on cross-work shift (pre- to post-) changes in MDA or 8-isoprostane was observed.

Conclusion: Urinary mutagenicity may serve as a suitable measure of occupational smoke exposures among wildland firefighters, especially among those lighting fires for prescribed burns.

	Williams, R., D. DeMarini, L. Stankowski, Jr., P. Escobar, E. Zeiger, J. Howe, R. Elespuru, and K. Cross. Are All Bacterial Strains Required by			
ORD-	OECD Mutagenicity Test Guideline TG471	David		
028576	Needed? Mutation Research / Genetic	DeMarini,		
Adv.	Toxicology and Environmental Mutagenesis.	ord, nheerl,	Peer	
Notification	Elsevier Science Ltd, New York, NY, USA,	istd, gctb	Reviewed	N/A

Impact/Purpose Statement

The current OECD Test Guideline TG471 for genotoxicity testing recommends the use of at least 5 strains of bacteria to evaluate compounds for gene mutation, along with various mammalian cell assays to test for chromosomal mutation. These guidelines are used by EPA, FDA, and most regulatory bodies worldwide. However, these guidelines have not been updated for 20 years, and the present study evaluated a large, 10,000 compound data base to assess whether all 5 bacterial strains are necessary to obtain sufficient determination as to the bacterial mutagenicity of a compound. As determined by this analysis, only 2 bacterial strains appear to be sufficient, when used in combination with the recommended mammalian cell assays for chromosomal mutation, such that 99% of mutagens will be detected. If this modification of the Test Guideline were adopted by OECD, and ultimately by EPA, FDA, and other regulatory bodies, there would be considerable reduction in the cost, time and redundancy to industry to comply with such mutagenicity test guidelines.

Product Description/Abstract

The International Workshop on Genetic Toxicology (IWGT) meets every four years to reach consensus recommendations on difficult or conflicting approaches to genotoxicity testing based upon experience, available data, and analysis techniques. The IWGT met in November 2017 in Tokyo, Japan, with one

working group tasked to assess the sensitivity and selectivity of the bacterial strains specified in the Organization for Economic Cooperation and Development (OECD) Test Guideline TG471 and to recommend possible modification of the test guideline. Initially, three questions were posed: (1) Although TA100 is derived from TA1535 and generally detects more mutagens than TA1535, does TA1535 detect any mutagens that are not detected by TA100? (2) Because there is a choice to use one of either Salmonella TA1537, TA97 or TA97a, are these strains truly equivalent? (3) Because there is a choice to use one of either E. coli WP2 uvrA, E. coli WP2 uvrA pKM101, or Salmonella TA102, are these strains truly equivalent? To answer these questions, we analyzed published bacterial mutation data in multiple strains from large (>10,000 compound) databases from Leadscape and Lhasa and anonymized data for 53 compounds in TA1535 and TA100 provided by a pharmaceutical company. Our analysis involved (1) defining criteria for determining selective responses when using different strains; (2) identifying compounds producing selective responses based upon author calls; (3) confirming selective responses by visually examining dose-response data and considering experimental conditions; (4) using statistical methods to quantify the responses; (5) performing limited additional direct-comparison testing; and (6) determining the chemical classes of compounds producing selective responses. We found that few mutagens (11) would fail to be detected if the test battery did not include Salmonella strains TA1535 (7), TA1537 (2), TA102, and E. coli WP2 uvrA (2). Consequently, 93% of the mutagens detected by the full TG471 strain battery were detected using only strains TA98 and TA100; addition of a clastogenicity assay increased this to 99%. Modification of testing based upon these findings would reduce the cost, time, and redundancy of the current OECD Test Guideline TG471.

	Bollman, M., G. DeSantis, R. DuChanois, M. Etten-Bohm, D. Olszyk, J. Lambrinos, and P. Mayer. Optimizing hydrologic performance of green roof media. ECOLOGICAL ENGINEERING. Elsevier Science Ltd, New York, NY, USA,	Paul Mayer, ord, nheerl, wed, eeb	Peer Reviewed	SSWR5.02A
ORD-027791				

Impact/Purpose Statement

Rainwater runoff in urban areas can cause localized flooding and deteriorate water quality, and urban landscapes can benefit from green infrastructure in a number of other ways. Installation of green roofs can address these issues, but optimal green roof design is critical for providing maximum benefits. Hydrologic performance of green roofs is strongly influenced by the composition of the growing media. In this study we measured hydrologic attributes (wet weight, water held at saturation, water retained after 14 days of drying, hydraulic conductivity) of several types of green roof growing media constituents, and predicted and tested the hydrologic performance of various mixtures of these constituents. Urban planners, building architects, and green roof designers can use the results of this study to assist in formulating optimal green roof media mixtures for specific applications. Maximizing the benefits of green roofs in urban areas furthers the Agency goals of ensuring clean water and improving living conditions in urban communities.

Product Description/Abstract

One of the primary functions of green roofs in urban areas is to moderate rainwater runoff, and one of the major impediments to the survival of plants on an extensive green roof (EGR) is a lack of available water during dry periods. Runoff moderation and water storage are both influenced by the composition of the growing media. In this three-phase study, we: 1) measured hydrologic attributes of individual EGR growing media constituents, 2) predicted hydrologic performance of media mixtures using data for the individual constituents, and 3) tested the seven top-ranking mixtures. Hydrologic attributes included wet

weight (WW), water held at saturation (WH), water retained after 14 days of drying (WR), and hydraulic conductivity (HC). We fixed the amount of organic matter (peat moss) at 20% by volume for media mixtures and varied the composition of the inorganic fraction. Because perlite was light in weight, yet held higher amounts of water both when saturated and after 14 days, media mixtures dominated by perlite were predicted to have the best overall hydrologic performance. Mixtures dominated by pumice were also predicted to perform relatively well, but were heavier. Although vermiculite retained the most water after its first wetting, its water retention sharply decreased after undergoing a drying and re-wetting cycle due to a decrease in volume of about 50%. Performance of tested mixtures reflected predictions for WW, WH, and WR; indicating performance of mixtures can be adequately predicted using performance of individual constituents for these attributes. Hydraulic conductivity (HC) was less predictable, possibly because the pore volume in mixtures may be unrelated to the pore volume of the individual constituents. Our approach is intended to provide a framework to test other media mixtures. The results of this study can be used to assist in formulating green roof media mixtures for specific applications, but also underscore the need for standardization of methods for more effective comparisons of EGR substrates as well as other attributes of EGRs, and reinforce the need to evaluate EGR components using real-world settings or scenarios.

	Figary, S., and N. Detenbeck. Comparing aquatic macroinvertebrate responses to impervious cover in Mediterranean climates using permutations and threshold analysis.	Naomi Detenbeck,	
ORD-027856	Freshwater Science. The Society for Freshwater Science, Springfield, IL,	ord, nheerl, aed, wdb	Peer Reviewed SSWR5.02A

Impact/Purpose Statement

Aquatic communities in streams can vary in their sensitivity to development based on geographic region, stream class and weather regime. This study compared the sensitivity of aquatic insect communities to impervious cover between the South Coast and Chapparal regions in California. California has a Mediterranean climate with large interannual variation in weather, so aquatic insect response was also examined between relatively wet, dry and average years. Sensitivity was also compared between streams with intermittent versus permanent flow. This study found that thresholds of impairment (rapid shifts in species composition) for imperviousness were very low and did not vary among different weather regimes. However thresholds of impairment were significantly higher for South Coast streams with permanent flow, probably because these streams were in areas of high human population densities with fewer pollution intolerant species present. This information will be critical when evaluating the effectiveness of green infrastructure stormwater management practices such as bioretention basins, green roofs, rain gardens, and permeable pavement in protecting stream condition.

Product Description/Abstract

Impervious cover (IC) has an inverse relationship to macroinvertebrate community diversity, but the % IC in a watershed that negatively impacts the community varies across regions. This study examines the macroinvertebrate response to % IC using Threshold Indicator Taxa ANalysis (TITAN) in the Mediterranean climate of California before a new California state General Construction Permit with more stringent stormwater management requirements was implemented. This study introduces a new method for comparing TITAN results using permutations and uses this method to determine variable macroinvertebrate sensitivity to % IC across Palmer Drought Severity Index (PDSI) categories, stream type (intermittent or perennial), and regions (Chaparral or South Coast). Additionally, this study

investigates land use and macroinvertebrate community composition differences between the regions and stream types. There were no significant differences among the macroinvertebrate responses to PDSI categories. The Chaparral streams and South Coast intermittent streams taxa that declined with increasing % IC had ecological thresholds between 0.08 – 0.43% IC, while the South Coast perennial streams had an ecological threshold between 1.62 - 2.94% IC. The South Coast perennial streams had a significantly higher threshold, using permutations, compared to the South Coast intermittent and Chaparral perennial streams. A TITAN sensitivity analysis was used to confirm that the distribution of sampling sites along the % IC gradient were not influencing the observed change points. Further investigation into the land use and macroinvertebrate community composition differences found that the South Coast perennial streams had higher human population densities in the region, lower % forested areas, and fewer pollution intolerant species, suggesting the higher ecological threshold could be from a widespread loss of pollution intolerant species in the region and stream type. Overall, the macroinvertebrate communities in the Chaparral region and South Coast intermittent streams were very sensitive to % IC, highlighting the need for extensive stormwater management in this region.

	Hagy, J., K. Houghton, D. Beddick, J. James, S. Friedman, and R. Devereux. Quantifying Stream Periphyton Community Responses to Nutrient Amendments using a Molecular Approach.	Jim Hagy,ord, nheerl, ged, edeb	Peer Reviewed	SSWR4.02A
ORD-028316	Freshwater Science. The Society for Freshwater Science, Springfield, IL,			

Impact/Purpose Statement

The objectives of this study were to investigate relationships between land use and water quality in streams, characterize periphyton community responses to water quality, and develop new sensitive and nutrient-specific periphyton indicators of water quality using a molecular approach based on analysis of DNA sequences. The most important and novel aspect of the research was use of DNA sequences to characterize microbial community composition of periphyton and develop indicators, providing an alternative to traditional approaches using microscopy. The manuscript should be of interest to scientists and water quality managers interested in methods for evaluating biotic condition in streams, specifically in relation to nutrient pollution. The manuscript should be especially of interest to those working to advance application of molecular methods evaluating stream periphyton communities.

Product Description/Abstract

Nutrient (nitrogen and phosphorus) pollution is a pervasive water quality issue in the US, including for small streams and rivers. Biotic condition of streams with respect to nutrients has been evaluated using biological indicators such as macroinvertebrate assemblages or periphyton communities, particularly diatoms. Molecular approaches have shown promise for this application, potentially because periphyton are incredibly diverse and their composition outside of diatoms, soft-bodied algae, or any single group may convey a wealth of information on responses to nutrients. To develop this further, we examined prokaryote and eukaryote community composition using 16S rRNA genes and 18S rRNA genes, respectively, and diatoms specifically. We examined periphyton nutrient responses in situ via experiments utilizing glass fiber filters that allowed diffusion of amended nutrients. Experimental setups were deployed in two southeastern US streams differing in the extent of agricultural land-use in the catchments. Experiments consisted of controls, nitrogen amendments (+N), phosphorus amendments (+P), and both N and P amendments (+NP). Periphyton communities that grew on the filters differed

significantly by stream, date or season, and nutrient treatment, with more consistent community differences observed among prokaryotes compared to eukaryotes. Effects of nutrient amendments were more pronounced in the less nutrient-impacted stream compared to the stream in an agriculture-dominated watershed. Combined +NP amendments decreased prokaryote species richness by 36% with similar declines in diversity. Significant associations of prokaryotes, eukaryotes and diatoms with controls or nutrient amendments were evaluated using indicator species analysis and used to compute indicators of nitrogen and phosphorus exposure. The resulting measures provided significant responses to nutrient amendments across sites and seasons. N vs. P effects were not well-resolved by broader community composition, but diatoms more responsive to P than N. Our results were encouraging in the context of existing studies, while also pointing toward further work needed to validate useful and practical molecular-based nutrient response indicators. This type of molecular approach may be of interest to environmental agencies and stakeholders responsible for assessing and managing stream water quality and biotic condition.

ORD-025751	Furey, R., M. ten Brink, J. Twichell, and I. Heilke. SystemSketch Fact Sheet. EPA/600/F-18/125	Marilyn Tenbrink, ord, nheerl, aed	Technical Fact Sheet	SHC 1.1.1.2
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Purpose/Impact Purpose

SystemSketch is a visualization tool that helps stakeholders better understand the context in which they are making decisions. It is web-based and interactive, and allows users to customize sketches to represent their decision context. Whether you are an individual or a community, making decisions can be a challenging process. Environmental management decisions present a unique set of challenges because outcomes have environmental, social, and economic implications. Understanding the implications of a decision is an important step of the decision-making process. Often, decision-making processes aren't able to account for the impacts on more than one domain because decision-makers lack access to the right information. SystemSketch provides this information and enables users to visualize the potential environmental, social, and economic outcomes of a decision. The SystemSketch Fact Sheet provides an overview of the SystemSketch tool. The fact sheet is intended for a wide audience, and provides information on the how the tool works, the content it contains, and the ways it can be used to support environmental management decisions. Additionally, this fact sheet provides specific examples of the data within SystemSketch and how it can be applied.

Product Description/Abstract

SystemSketch is a visualization tool that helps stakeholders better understand the context in which they are making decisions. It is web-based and interactive, and allows users to customize sketches to represent their decision context. This Fact Sheet provides an introduction to SystemSketch by (1) describing the functions within the tool and (2) showing how the tool can be used to better understand a decision context. SystemSketch enables users to understand causal relationships in any kind of natural resource management setting. Users can work backwards to understand what caused something to happen, or they can look forward to see probable future effects. The tool provides an intuitive way for users to approach decisions from an informed, interdisciplinary perspective, while making evident the links between ecosystem services and human benefits. SystemSketch is both a scoping tool that explores a decision context and an informational resource that helps guide users through the beginning stages of a structured decision making (SDM) process. Users explore environmental, social, and economic concepts and their relationships and use these factors to build interactive conceptual maps.

They can then use their selected sketches to identify community objectives, clarify management options, and investigate useful indicators. The SystemSketch Fact Sheet provides an overview of the tool. The fact sheet is intended for a wide audience, and provides information on the how the tool works, the content it contains, and the ways it can be used to support environmental management decisions.